

1732-Pos Board B624**Understanding High Definition Immunoassays (HDIA): Studies of Binding Kinetics on Microparticles**

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Fluorescence imaging provides both spatial and temporal information about target molecules in biological systems. We have proposed to use imaging as a detection technique in microparticle based immunoassays. It has advantage over traditional approaches which measure only total signal but do not make use of the spatial or temporal information embedded in the system. The method can be readily adapted for quick assay prototyping and high throughput screening on any conventional fluorescence imaging system.

In microparticle immunoassays, analytes are captured with antibody coated microparticles and subsequently detected using second antibody labeled with a reporter group. In HDIA, fluorescence images of the microparticles are examined pixel-by-pixel to extract binding information only from the microparticles, thus minimizing irrelevant signals from solution and vessel surfaces. Our model systems include sandwich based Troponin and Bcl-xl (B-cell lymphoma-extra large) protein assays, as well as homogeneous competitive Methotrexate immunoassay.

Performance of the HDIA is dominated by binding kinetics of the microparticles. Depending on the microparticle number, concentration of the binding sites on microparticles, sample volume, sample concentration and the geometry of the reaction vessel, the binding kinetics can be either reaction limited or diffusion limited. We will present the effects of these factors on the binding kinetics of HDIA.

1733-Pos Board B625**EPSP Amplitudes and Dynamics in Dendritic Spines using Voltage-Sensitive Dyes**

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Dendritic spines are the initial site of the processing of information carried by excitatory network activity. Spine morphology often includes a narrow neck, which is known to isolate the biochemical signaling components within the spine from the dendritic branch. To what degree spines are isolated electrically and how local excitatory postsynaptic potentials (EPSPs) behave is still not fully understood. It is known, however, that the larger the electrical isolation, the larger the amplitude of membrane potential changes, which may have a significant impact on signaling mechanisms within the spine responsible for important processes such as synaptic plasticity. We are addressing this question using 2-photon imaging of voltage sensitive dyes along with synaptic activation via glutamate uncaging in acute brain slices. Using a custom microscope we can control the positions of both a recording laser (fixed at the center of a spine) and the uncaging pulse directed at the desired uncaging position just off the spine. The amplitudes of the optical signals in the spine are calibrated using backpropagating action potential waveforms, which, as previously described, is consistent across spines of different sizes and shapes. Uncaging-evoked EPSP amplitudes are typically 10mV-20mV, which are highly attenuated upon reaching the soma, where they typically appear 20 fold smaller in amplitude. Additionally, EPSP dynamics within the spine are typically much faster in the spine, with half-widths around 5-10ms, compared to the same EPSPs measured at the soma, which are typically 50-100ms. The observed attenuation of EPSP amplitudes and increase in duration were used to fit a biophysical, NEURON-based model, in order to explore the role of the spine geometry. NIH grants R01 EB001963 and P41 GM103313

1734-Pos Board B626**Calcium Imaging and Optical Manipulation of Neuronal Activity in Axolotl using a LED-Based Microscope**

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Here we introduce a novel fluorescence microscope concept which expands the application of functional fluorescence imaging and further enables optical manipulation of biological samples. Taking advantage of the LED technique allows a space saving housing of multiple light sources and illumination at various wavelengths. Furthermore, the microscope provides an optical separation into two independently controllable excitation pathways enabling a simultaneous illumination of different regions which are adjustable in size. Image detection is realized via a super resolution high speed camera that allows high resolution image capturing (2560x2160) at rates up to 100 Hz. Moreover, we implemented a voice-coil driven high NA objective to ensure z-movement at

maximum speed and precision. The multiple LED arrangement and the separation of excitation pathways therefore facilitate the usage of multiple imaging approaches at the same time.

To test the performance of the microscope we used *in vitro* whole head preparations of *Ambystoma mexicanum* tadpoles. This preparation allows studying neuronal systems with all sensory pathways intact and can be maintained up to one week. Thus, we were able to use calcium imaging to record sensory evoked neuronal responses of central vestibular neurons elicited by electric stimulation of specific semicircular canals. Further, to quantify the glutamate uncaging efficiency we calculated the required light intensity and duration to optically evoke action potentials by patching those neurons. Finally, we could show that by means of spatially separating calcium imaging and glutamate uncaging we were able to manipulate ipsilateral semicircular canal evoked calcium responses by optically activating contralateral inhibitory/excitatory pathways.

1735-Pos Board B627**Optimization of Mitochondrial and Cytosolic pH Determination in Madin Darby Kidney Cells using CLSM Images**

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Reactive Oxygen Species (ROS) production and impairment of mitochondrial functioning is a central research topic. A recent review for Multiple Sclerosis¹. Investigation of mitochondrial functioning in living cells under normal physiological conditions and metabolic inhibition provides biologically relevant information using confocal cell imaging^{2,3}. Cytosolic calcium clearance does not necessarily lead to mitochondrial dysfunction as long as the mitochondrial matrix pH stayed acidified compared to the cytosol.² Studying intra-mitochondrial versus cytosolic electrolyte homeostasis in living cells under metabolic stress might be crucial to understand the conditions where mitochondria become detrimental by producing ROS. Caveats are presented to properly obtain information about mitochondrial and cellular pH, structure and properties of the mitochondrial network under normal conditions and metabolic inhibition, by using optimized protocols⁴. The method critically depends on availability and selection of nuclear areas with lowest background fluorescence contribution while keeping experiment conditions rigorously similar. We present the influence of factors such as cell type, plating number, age of the cells, attachment surface properties, cell confluency, loading protocol of the dyes, background area selection on the values obtained. Optimization of parameters such as objective choice, detector voltage stability, fluorescence intensity saturation, illumination power and bleaching, imaging depth, point spread function (PSF) and reduction of cell motility are elucidated. Confluent Madin Darby Canine Kidney (MDCK) cells were loaded with mitotracker green and pH indicator SNARF. Images were collected with a Zeiss LSM 510 Meta CLSM. Data were analyzed with ImageJ⁴, and Matlab.

1. Lassmann, H., and van Horssen, J., FEBS Lett. 2011

2. Smets, I. et al., Am. J. Physiol. Renal Physiol., 2004

3. Baron, Sz. et al., JASN, 2005

4. vandeVen, M. & Balut, C. et al. Methods Mol Biol. 2010

1736-Pos Board B628**Application of FTIR Imaging on Healthy (Donor Age Effect) and Disease (Beta Thalassemia Major) States**

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Recent researches have displayed the significant role of stem cells in tissue renewal and homeostasis with their unique capacity to develop different cell types. Mesenchymal stem cells (MSCs) which are non-hematopoietic, stromal cells found primarily in bone marrow (BM) in addition to many human tissues. Bone marrow mesenchymal stem cells (BM-MSCs) can differentiate into a variety of non-hematopoietic tissues and maintain healthy hematopoiesis by providing supportive cellular microenvironment in BM. Stem cell studies hold enormous potential for development of new therapies. Therefore; investigation of stem cells in normal developmental and physiological states as well as in pathological conditions may lead to understanding of disease pathogenesis and development of new cellular therapies. The present study focused on the investigation of donor age effect on healthy human BM-MSCs and the characterization of beta thalassemia major (β -TM) disease by using a novel, rapid and non-destructive technique, Fourier transform infrared microspectroscopy (FTIRM). Aging process of healthy BM-MSCs is significant because of their important role in tissue regeneration and repairment. Characterization of